The Influence of Soil Topography and Spore-Rain Density on Gender Expression in Gametophyte Populations of The Homosporous Fern *Aspidotis densa*

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The gametophytes of homosporous pteridophytes, being free-living and independent of the sporophyte, experience an immediacy with the external environment unique among the gametophytes of vascular plants. As a result, the environment directly influences the demographics of homosporous fern gametophytes, particularly their distribution, development, and mating systems. Because most homosporous fern gametophytes are photosynthetic, require light to germinate, and develop gametangia sequentially, environmental factors that alter the quantity and quality of light to neighboring spores and gametophytes may stimulate asynchronous germination, growth, and sexual maturation. The influence of environmental factors upon the population biology of fern gametophytes may be augmented by the production of antheridiogens. The meristematic gametophytes of some species have been shown to produce antheridiogens or as yet unspecified metabolites that generate "antheridiogen effects" (induced germination in darkness, precocious maleness, and delayed development of a notch-meristem) in ameristic neighbors (Näf, 1963, 1975; Schedlbauer & Klekowski, 1972; Hamilton, 1989; Haufler & Gastony, 1989; Schneller, et al., 1990). In some species, individuals with notch-meristems are insensitive to antheridiogen (Näf, 1963). In such species, asynchronous growth and development among neighbors is essential for an antheridiogen to influence the gender composition of a population.

Microvariation in soil topography may alter the availability of light to spores and their ensuing gametophytes. Spores shed on well-illuminated regions of a heterogeneous soil profile are likely to germinate early compared to less-illuminated neighbors. The resulting gametophytes will mature rapidly, and through the action of an antheridiogen, influence the germination and sexual expression of neighbors in poorly illuminated regions.

Because antheridiogens are water soluble and of uncertain stability in the environment (Näf, 1963), and because homosporous fern gametophytes require water for fertilization, the proximity of neighboring gametophytes may be another important determinant of gender composition and mating systems. For example, the probability that colonizing gametophytes are reproductively isolated is associated with a higher probability of selfing, resulting in genetically homozygous progeny without genetic load (Lloyd 1974a, 1974b; Crist & Farrar, 1983; McCauley, et al., 1985; Soltis, et al., 1988; Peck, et al., 1990; Holsinger, 1991; Watano & Masuyama, 1991). Alternatively, in taxa producing dense gametophyte populations, close proximity may facilitate interactions between neighbors, leading to outcrossing mating systems, high levels of genetic load, and heterozygous sporophytes (Lloyd, 1974a, 1974b, 1988; Singh & Roy, 1977; Duckett & Duckett, 1980; Soltis & Soltis, 1986, 1987; Soltis, et al., 1988; Holsinger, 1991). Thus, by decreasing distances between neighbors, dense spore-rain may amplify the influences that asynchronous germination and development have on the sexual expression and mating system of a population and low densities of spore-rain may result in distances too large for interactions among gametophytes (Cousens & Horner, 1970; Tryon & Vitale,

1977; Cousens, 1979; Von Aderkas, 1983; Rubin, et al., 1985; Klekowski & Lloyd, 1968; Lloyd, 1988; Schneller, 1988; Peck, et al., 1990; Hamilton & Lloyd, 1992). This study was designed to determine the influence of soil topography and spore-rain density on the gender composition of gametophyte populations of *A. densa* (Brack. in Wilkes) Lellinger.

A. densa is an endemic of serpentine rock outcrops of western North America. Its range extends from British Columbia south to California and east to Montana and Utah (Wagner, 1957; Smith, 1975; Lellinger, 1985). Gametophytic ontogeny is regulated by an antheridiogen system (Greer, 1991). In isolation, gametophytes develop into meristematic, archegoniate individuals that remain unisexual for more than six months. Antheridia occur only on ameristic gametophytes in multispore cultures or on gametophytes grown on agar that supported a previous generation of meristematic gametophytes. Insensitivity to antheridiogen correlates with the development of a notch-meristem. The antheridiogen of *A. densa* also induces spore germination in darkness.

METHODS AND MATERIALS

Fertile fronds were collected from five sporophytes at each of the three following California populations of *A. densa:*

1. Alpine lake, Marin Co., south face of Azalea Hill below water tank summit on Bolinas-Fairfax road. T1N, R7W.
2. Horse Mountain, Humboldt Co., south facing slope along summit road; 1/4 mile southeast beyond radio tower. T6N, R4E, S28.
3. Bridge over north fork of Feather River, 1/4 mile south of Pulga city, State Hwy 70, Butte Co., serpentine outcrop above southeast end of bridge. T22N, R5E, S6.

To investigate the influences of soil topography and spore-rain density on the gender composition of gametophyte populations, two soil textures, "fine" and "coarse", were each combined with two spore-rain densities, "low" and "high", to produce a 2 X 2 set of treatments (Table 1). Each treatment combination was replicated across fifteen sporophytes. Gametophyte populations were established, cultured, and observed for gender composition as described below.

Serpentine soil (from the top 5 inches of the soil horizon) was collected from Horse Mtn., Humboldt Co.; and Kneeland airport, Humboldt Co., – both sites of vigorous *A. densa* populations. A 50:50 mixture, by volume, of the two soils was prepared, and the composite sieved into the following size classes: 1) 4.0mm < X < 15.6mm, 2) 1.0mm < X < 4.0mm, and 3) X < 1.0mm. The fine-grained soil was prepared using equal proportions, by weight, of soil classes 2 and 3. The coarse-grained soil was prepared by mixing soil classes 1 and 3 at a weight ratio of 87:13 respectively. Each soil sample was wetted with distilled water, transferred into sterile 100mm x 20mm glass petri dishes until approximately 1/2 full, and autoclaved.

Spores from each sporophyte were sifted to remove sporangia and debris, and allotments were weighed. Low density treatments averaged 0.0073g (± 0.0005g) per replicate, and high density treatments averaged 0.0638g (± 0.0005g) per replicate. To create an even distribution of spores, the spores for each treatment were placed in two 100mm x 20mm glass petri dish lids, and randomized by pouring the spores into one lid, overturning, and rotating while tapping above the other. The procedure was repeated until the spore counts among each of 5 random fields of view in a Wild M5A stereomicroscope deviated by no more than 20 percent. Each spore-rain density treatment was then inoculated on to each soil texture treatment by overturning and tapping the lid.

Table 1. Pooled data (mean ± SD) for gametophyte density and gender for fine vs. coarse soil texture and low vs. high spore-rain density treatments.

Soil Texture/ Spore-Rain Density	Gametophytes/ 1.25cm^2	Gender			
		Male	Female	Bisexual	Vegetative
Fine/Low	46.56	171	540	11	472
	(±41.5)	(14.3%)	(45.2%)	(1%)	(39.5)
Fine/High	215.3	716	401	0	468
	(±149.3)	(45.2)	(25.3%)	-	(29.5%)
Coarse/Low	33.2	312	587	2	441
	(±24.5)	(23.3%)	(43.8%)	(<0.01%)	(32.9%)
Coarse/High	370.8	941	291	1	372
	(±291.5)	58.7%)	(18.1%)	(<0.01%)	(23.2%)
Fine	129.9	887	911	11	940
	(±140.3)	(32.3%)	(33.1%)	(<0.01%)	(34.2%)
Coarse	211.56	1399	878	1	813
	(±271.1)	(45.3)	(28.4%)	(<0.01%)	(26.3%)
F = 3.83, p = 0.055					
Low	33.8	485	1127	13	913
	(±26.4)	(19.1%)	(44.4%)	(<0.01%)	(36.%)
High	305.6	1657	662	1	840
	(±249.4)	(52.4%)	(21%)	(<0.01%)	(26.6%)
F = 32.13, p = 0.000					

All treatments were placed in clear plastic bins lined with moist paper towel to prevent desiccation. Each was maintained in a culture chamber at a constant temperature of 21°C (± 2°C), and illuminated at an irradiance of approximately 60 microeinsteins/sec/cm^2 under a 12/12 hour light/dark regimen using equal banks of warm and cool white 40 watt fluorescent lights.

Each treatment dish was observed regularly using a Wild M5A stereomicroscope, and patterns of germination, relative size, and morphology noted. After 12 weeks of culture, 5 random 12.5mm^2 samples were removed from each treatment dish, the gametophytes washed free of debris, separated, and counted at 25x and 50x using a Wild M5A stereomicroscope. A minimum of one hundred gametophytes were randomly selected, observed at 40x and/or 100x using a compound light microscope, and scored for morphology and gender expression.

A two-way Analysis of Variance (Sokal & Rohlf, 1981) was used to analyze the relationship between each treatment (soil texture and spore-rain density) and the percentage of males in the population.

RESULTS

Gametophyte density corresponded with spore-rain density. Mean gametophyte density for populations established by low spore-rain density was 33.8 (±26.4) gametophytes per 1.25cm^2, and 305.6 (±249.4) gametophytes per 1.25cm^2 for populations established by high spore-rain density. Determination of gametophyte density in populations established by high spore-rain density was complicated by profuse branching of filamentous and spatulate gametophytes. Consequently, estimates of gametophyte density in these populations are considered crude approximations.

The frequency of males in gametophyte populations of A. densa increased significantly under the influences of coarse-grained soil (45.3% male; F = 3.83, p = 0.055), and high spore-rain density (52.4% male; F = 32.13, p = 0.000) (Table 1). The fewest males

(14.3%) occurred in the paired treatments of low spore rain density and fine-grained soil. Germination and sexual maturation of gametophytes appeared to be nearly synchronous, resulting in predominantly female populations. The greatest number of males (58.7%) occurred in the pairwise treatments of high spore-rain density and coarse-grained soil (Table 1). Germination and sexual maturation of gametophytes in populations established by high spore-rain and/or on coarse-grained soil appeared to be asynchronous. Gametophytes on the upper 1/2 of the soil profile were well-illuminated, first to mature, and predominantly female, whereas those in less-illuminated sites were smaller (often filamentous) and predominantly male.

DISCUSSION

The bigametophytic ontogeny and antheridiogen system of *A. densa* established an obligate cross-fertilizing mating system that virtually precludes intragametophytic selfing (Greer, 1991). Because gametophytes with notch-meristems both produce, and are themselves insensitive to, antheridiogen, asynchronous development among neighbors is essential for the establishment of populations of mixed gender. Thus, factors that promote asynchronous germination and development are important determinants of gender composition, fertilization success, and mate competition in populations of *A. densa*.

Both spore-rain density and soil topography influence asynchronous development and the gender composition of gametophyte populations of *A. densa*. I hypothesize that the heterogeneous topography imparted by coarse-grained soil partitions the availability of light to disseminated spores, providing a basis for asynchronous germination, growth, and development. Spores in well-illuminated sites germinate early. The ensuing gametophytes develop rapidly and are the first to reach sexual maturity. Spores in less-illuminated sites fail to germinate or germinate late, and the ensuing gametophytes develop slowly. As a result, their sensitivity to antheridiogen from better illuminated, early maturing neighbors is prolonged.

Increasing spore-rain densities may facilitate the action of antheridiogen by reducing distances between neighbors (neighborhood size). The difference between the mean neighborhood size of low density populations (4.62 sq.mm) and high density populations (0.51sq.mm) was only 4.11sq.mm, suggesting that antheridiogens may influence the germination, growth, and gender of neighbors only within very limited distances. Although the horizontal diffusion of antheridiogen within agar may be considerable (Voeller, 1969), horizontal diffusion within soil may be greatly limited by factors such as extremes in soil moisture content and the gravitational movement of soil water. Furthermore, the stability of antheridiogen within soil is completely unknown. Neighborhood size may be particularly important among gametophytes receiving equivalent illumination, where the influence of antheridiogen may also be restricted by the nearly synchronous development of neighbors.

If antheridiogen activity occurs only within very limited distances, fertilization success will be most likely, and male mate-competition most severe, in *A. densa* populations established on sites with heterogeneous microtopography, and/or by moderate to high densities of spore-rain (as might be expected near the spore source or in sites favored by wind deposition). Conversely, fertilization success may be severely limited and female mate-competition most severe in populations established on sites with little microsite relief, and/or by low densities of spore-rain. In populations established by very low densities of spore-rain, which can occur within a few meters from the source (Peck, et al.,

1990), spore longevity and the accumulation of spore banks would be particularly important factors determining fertilization success.

Factors such as soil topography and spore-rain density that promote asynchronous germination, growth, and development among neighboring gametophytes may indirectly influence the survival of sporophytic progeny. The earliest maturing gametophytes, such as those growing in well-illuminated sites, are likely to produce the first progeny of the reproductive season. The timing of reproduction during the growing season may be one of the most important events predicting progeny survival. This could be particularly true in dense gametophyte populations, where large numbers of progeny are likely to compete for limited space. Progeny produced earliest in the growing season would have distinct competitive advantage in resource capture and establishment. Moreover, progeny produced early in the growing season, regardless of cohort densities, may be more likely to develop to stress tolerant stages before the onset of "nongrowing" seasons. As a fern endemic to serpentine outcrops, where safe sites appropriate for the gametophyte and juvenile sporophyte are greatly restricted, unevenly distributed, and heterogeneous, the gender composition, mate-competition, fertilization success, and progeny survival of *A. densa* populations should vary considerably from site to site.

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